CHAPTER 7

AGRI-SCREEN TEST KIT

7.1 GENERAL INFORMATION

The Agri-Screen test is a sequential competitive enzyme immunoassay that provides qualitative (equal to or less than a specified threshold) results.

7.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Agri-Screen test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

7.3 EXTRACTION PROCEDURES

a. Standard Procedure.

(1) Transfer 50 grams of ground sample into an extraction mixing jar.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent.

(3) Cover the extraction jar and blend on high speed for 2 minutes.

(4) Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.

(5) After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

b. Alternate Procedure.

(1) Transfer 50 grams of ground sample into a whirlpack bag.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent to the bag and secure tightly.

(3) Shake the sample portion and extraction solvent vigorously by hand for 3 minutes.

(4) Let the slurry stand for 1 minute, then pour off a small amount of the extract from the bag into the filter paper mounted over the collection container.

(5) Close the bag securely and save until ready for waste disposal.

7.4 TEST PROCEDURES

a. Preparation of Solutions.

(1) Remove aluminum seals from blue-labeled, red-labeled, and yellow-labeled bottles and set aside.

(2) Substrate is pre-activated and is ready for use. Substrate should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

b. Sample Analysis.

(1) Select a test kit that has stabilized/warmed to room temperature 68° - 82° F for 1-hour prior to use.

(2) Open a foil bag and remove 2 red-marked mixing wells and 2 antibody-coated wells for each sample to be tested. Place in the microwell holder and mark the left end of the strip with a "1". Return unused strips to the foil package and close tightly.

Document the identification of all antibody-coated sample wells in order to identify the wells after washing.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C	S1	C	S2	C	S3	C	S4	C	S5	C	S6

"W" = well number e.g., #1 through #12
"C" = control
"S1, S2, S3, & S4" = sample numbers

Where C is the 20 ppb control, and S1, S2, etc. are the sample numbers

(3) Firmly place a pipette tip on the 100 microliter (µl) pipettor/syringe and add one preset amount of the Enzyme Conjugate (blue-labeled bottle) into each mixing well (priming pipette tip first). Discard pipette tip.

NOTE: When dispensing any test liquids, prime pipette tip by drawing liquid up into the tip and dispensing it back into the bottle one or two times.

(4) Firmly place a pipette tip on the 100 µl pipettor/syringe and add one preset amount from the 20 ppb Control (yellow-labeled bottle) to the first mixing well of the red-marked strip. Discard the pipette tip.

If testing more than one sample, place 100 µl of 20 ppb Control into mixing well #3 (second sample), mixing well #5 (third sample), etc.

(5) Firmly place a new pipette tip on the 100 µl pipettor/syringe and add one preset amount from the filtered extract in the second well of the red-marked mixing strip. Discard tip.

(6) Repeat step (5) for each subsequent sample using the designated sample wells and new pipette tip for each.

NOTE: If using a single channel pipettor or syringe, steps (5) through (8) must be performed individually and as quickly as possible.

(7) Using a pipettor/syringe, mix the contents of the mixing wells by pipetting up and down in the tips 5 times.

(8) Using a new tip for each well, transfer 100 µl from each mixing well to the corresponding antibody-coated well. Discard red mixing wells and used pipette tips.

(9) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

(10) Set the timer and allow the antibody-coated wells to incubate for 5 minutes.

(11) After the incubation reaction is complete, shake out the contents of the antibody-coated wells.

(12) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and dump out. Repeat this step 10 times.

(13) Remove all water droplets by turning wells upside down and gently tapping over a paper towel until all of the water is removed.

(14) Firmly place a new pipette tip on the pipettor/syringe and transfer 100 µl of substrate (green-capped tube) to each antibody-coated well. Discard tip.

(15) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

(16) Set the timer and allow the antibody-coated wells to incubate for 5 minutes.

(17) After the incubation reaction is complete, firmly place a new pipette tip on the pipettor/syringe and transfer 100 µl of red stop solution (red-labeled bottle) to each antibody-coated well. Discard tip.

(18) Mix the antibody-coated wells by gently sliding the microwell holder/wells back and forth on a horizontal surface for 10 to 20 seconds. Be careful not to splash solution out of wells.

c. Interpreting Results.

Place the well strip on a white surface when determining results. Interpret the test results as follows:

(1) Equal to or less than 20 ppb.

The sample is considered equal to or less than 20 ppb when the "Sample" well is as blue or darker (blue) than the control well.

(2) Greater than 20 ppb.

The sample is considered greater than 20 ppb when the "Sample" well shows less blue color (more red color) than the control well.

7.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

b. Certify results as being equal to or less than a threshold.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

7.6 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

7.7 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

7.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) Foil pouch with 24 antibody-coated wells and 24 red mixing wells.

(2) 1 yellow-labeled bottle of 20 ppb aflatoxin control solution.

(3) 1 blue-labeled bottle of enzyme conjugate solution.

(4) 1 green-labeled bottle of substrate solution.

(5) 1 spring syringe.

(6) 75 pipette tips.

b. Materials Required but not Provided:

(1) Timer (5 minute capacity).

(2) 100 µl pipettor (single or multi-channel) with tips.

(3) Microwell holder.

(4) Wash bottle.

(5) Felt tipped pens.

(6) Balance.

(7) Sample Grinder.

(8) Methanol - ACS grade or better.

(9) Deionized or Distilled Water.

(10) Blender with mixing jars.

(11) Whatman No.1 Filter Paper or Coffee Filters.

7.9 STORAGE CONDITIONS

a. Storage Conditions.

(1) Test kits should be refrigerated between 36E- 48EF.

(2) Do not freeze any of the kit components or expose reagents to temperatures greater than 95E F.

b. Precautions.

(1) Do not use kit components beyond expiration date.

(2) Do not use reagents from one kit with reagents from a different kit.

(3) Use of incubation times other than those specified may give inaccurate results.

(4) Avoid prolonged storage of kits at ambient temperatures.